

CADASIL Treatment With Cholinesterase Inhibitors

Related Applications

This application claims priority to US Application No. 60/549,939 filed March 5, 2004, the disclosure of which is incorporated by reference herein.

Field of the Invention

The invention provides methods for treating and/or preventing cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) by administering to patients safe and effective amounts of cholinesterase inhibitors.

Background of the Invention

Stroke is the third leading cause of death and the first cause of acquired physical or cognitive impairment in developed countries. Strokes are ischaemic in 80% of cases and the leading causes are atheroma and cardiac emboli. Despite extensive investigation, up to 40% of cases remain without definite etiology.

Familial causes of stroke have been identified, such as MELAS and homocystinuria. Since 1977, nine unrelated families have been reported with a mendelian syndrome that leads to stroke. A large pedigree was reported which allowed for the precise definition of the clinical, neuro-imaging and genetic parameters of this disease. This condition is characterized by recurrent subcortical ischaemic strokes and dementia. It is underlaid by a cerebral non-atherosclerotic, non-amyloid angiopathy affecting mainly the small arteries penetrating the white matter and basal ganglia. All reported families share strikingly similar clinical, neuro-imaging and pathological features. The acronym CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is used to describe this condition.

It has been shown that the human Notch3 gene is mutated in patients with CADASIL. Most of the mutations cause amino acid changes in the extracellular domain. Genetic linkage analysis conducted on two large CADASIL pedigrees assigned the CADASIL locus to chromosome 19 and multilocus analysis with the location scores method established the best estimate for the location of the gene within a 14 cM interval bracketed by D19S221 and D19S215 loci.

The invention is directed, among other things, to methods for treating and/or preventing CADASIL.

Summary of the Invention

The invention provides methods for treating and/or preventing cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) in a patient in need thereof by administering a therapeutically effective amount of at least one cholinesterase inhibitor. The methods may further comprise administering therapeutically effective amounts of HMG-CoA reductase inhibitors.

The invention provides methods for treating and/or preventing neurovascular diseases caused by one or more mutations of the human Notch3 gene by administering to a patient in need thereof a therapeutically effective amount of at least one cholinesterase inhibitor. The methods may further comprise administering therapeutically effective amounts of HMG-CoA reductase inhibitors.

The invention provides pharmaceutical compositions comprising therapeutically effective amounts of cholinesterase inhibitors, HMG-CoA reductase inhibitors, and pharmaceutically acceptable carriers.

The invention is described in more detail below.

Detailed Description of the Invention

The invention provides methods for treating and/or preventing cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) in a patient in need thereof by administering a therapeutically effective amount of at least one cholinesterase inhibitor. CADASIL is a neurovascular disease believed to be caused by one or more mutations of the human Notch3 gene. Clinical manifestations of CADASIL are described herein.

CADASIL can be diagnosed by analyzing the skin of a patient by electron microscopic examination. In a skin analysis, CADASIL is often marked by granular osmiophilic material within the membrane surrounding pericytes and smooth muscle cells of vessels of the skin. CADASIL can be diagnosed by magnetic resonance imaging (MRI) of the brain of the patient. In a brain analysis, CADASIL is often marked by subcortical and white matter alterations compatible with subcortical infarcts and demyelination, respectively. In other embodiments, CADASIL can be diagnosed through genetic testing for one or more Notch3 gene mutations.

In another embodiment, the invention provides methods for treating and/or preventing cerebral autosomal dominant arteriopathy with subcortical infarcts and

leukoencephalopathy (CADASIL) in a patient in need thereof by administering a therapeutically effective amount of at least one cholinesterase inhibitor and at least one HMG-CoA reductase inhibitor. The cholinesterase inhibitor and HMG-CoA reductase inhibitor can be administered separately or in the form of a pharmaceutical composition. Exemplary HMG-CoA reductase inhibitors include simvastatin, lovastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, and the like. The cholinesterase inhibitor and HMG-CoA reductase inhibitor can be in the form of a pharmaceutically acceptable salt.

The invention provides methods for treating and/or preventing neurovascular diseases caused by one or more mutations of the human Notch3 gene by administering to a patient in need thereof a therapeutically effective amount of at least one cholinesterase inhibitor. More particularly, the neurovascular diseases are caused by one or more mutations of the human Notch3 gene at the location bracketed by D19S221 and D19S215 loci. The cDNA sequence of the human Notch3 gene and the corresponding protein sequence are known in the art and described, for example, in US Patent No. 6,537,775, the disclosure of which is incorporated by reference herein in its entirety.

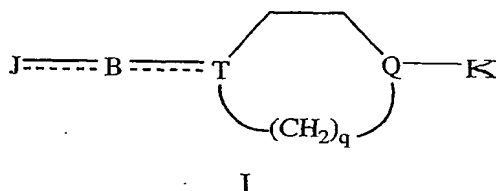
The point mutations of the Notch3 gene lead to the creation or to the disappearance of a cysteine in one of the EGF domains of the Notch3 protein. These mutations are clustered for a large part into the first six EGFs. The clustering of the mutations is important in diagnostic terms especially for the sequential search for these mutations. Moreover, all mutations lead to the presence of an odd number of cysteines in one of the EGFs (either seven or five cysteines) instead of the six cysteines normally present. These mutations could result in the formation of either intra- or intermolecular (and in this case in the formation of homo- or heterodimers) aberrant disulfide bridges.

In another embodiment, the invention provides methods for treating and/or preventing neurovascular diseases caused by one or more mutations of the Notch3 gene by administering a therapeutically effective amount of at least one cholinesterase inhibitor and at least one HMG-CoA reductase inhibitor. The cholinesterase inhibitor and HMG-CoA reductase inhibitor can be administered separately or in the form of a pharmaceutical composition. Exemplary HMG-CoA reductase inhibitors include simvastatin, lovastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, and the like.

The cholinesterase inhibitor and HMG-CoA reductase inhibitor can be in the form of a pharmaceutically acceptable salt.

The cholinesterase inhibitor used in the methods and pharmaceutical compositions of the invention can be any in the art. The cholinesterase inhibitor can be, for example, an acetylcholinesterase inhibitor or a butyrylcholinesterase inhibitor. Acetylcholinesterase inhibitors are preferred. Exemplary cholinesterase inhibitors include donepezil, phenserine, tolserine, phenethylnorcymserine, ganstigmine, epastigmine, tacrine, physostigmine, pyridostigmine, neostigmine, rivastigmine, galantamine, citicoline, velnacrine, huperzine (e.g., huperzine A), metrifonate, heptastigmine, edrophonium, TAK-147 (i.e., 3-[1-(phenylmethyl)-4-piperidinyl]-1-(2,3,4,5-tetrahydro-1H-1-benzazepin-8-yl)-1-propanone fumarate or other salts thereof), T-82, upreazine, and the like. In each of the methods described herein, one or more cholinesterase inhibitors can be used. In one embodiment, one cholinesterase inhibitor is used. In another embodiment, donepezil, a stereoisomer thereof and/or a pharmaceutically acceptable salt thereof and a second cholinesterase inhibitor are used in the methods or compositions of the invention.

In one embodiment, the cholinesterase inhibitor can be a compound of formula I, a stereoisomer thereof, and/or a pharmaceutically acceptable salt thereof:



wherein J is

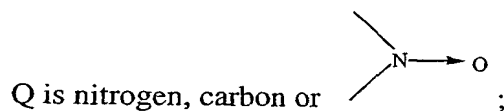
- (a) a substituted or unsubstituted group selected from the group consisting of (1) phenyl, (2) pyridyl, (3) pyrazyl, (4) quinolyl, (5) cyclohexyl, (6) quinoxalyl, and (7) furyl;
- (b) a monovalent or divalent group, in which the phenyl can have one or more substituents selected from (1) indanyl, (2) indanonyl, (3) indenyl, (4) indenonyl, (5) indanedionyl, (6) tetralonyl, (7) benzosuberonyl, (8) indanolyl, and (9) $C_6H_5-CO-CH(CH_3)-$;
- (c) a monovalent group derived from a cyclic amide compound;
- (d) a lower alkyl group; or

(e) a group of $R^{21}-CH=CH-$, in which R^{21} is hydrogen or a lower alkoxy carbonyl group;

B is $-(CHR^{22})_r-$, $-CO-(CHR^{22})_r-$, $-NR^4-(CHR^{22})_r-$, $-CO-NR^5-(CHR^{22})_r-$,
 $-CH=CH-(CHR^{22})_r-$, $-OCOO-(CHR^{22})_r-$, $-OOC-NH-(CHR^{22})_r-$, $-NH-CO-(CHR^{22})_r-$,
 5 $-CH_2-CO-NH-(CHR^{22})_r-$, $-(CH_2)_2-NH-(CHR^{22})_r-$, $-CH(OH)-(CHR^{22})_r-$,
 $=(CH-CH=CH)_b-$, $=CH-(CH_2)_c-$, $=(CH-CH)_d=$, $-CO-CH=CH-CH_2-$,
 $-CO-CH_2-CH(OH)-CH_2-$, $-CH(CH_3)-CO-NH-CH_2-$, $-CH=CH-CO-NH-(CH_2)_2-$, $-NH-$,
 $-O-$, $-S-$, a dialkylaminoalkyl-carbonyl or a lower alkoxy carbonyl;

wherein R^4 is hydrogen, lower alkyl, acyl, lower alkylsulfonyl, phenyl,
 10 substituted phenyl, benzyl, or substituted benzyl; R^5 is hydrogen, lower alkyl or phenyl;
 r is zero or an integer of about 1 to about 10; R^{22} is hydrogen or methyl so that one
 alkylene group can have no methyl branch or one or more methyl branches; b is an
 integer of about 1 to about 3; c is zero or an integer of about 1 to about 9; d is zero or
 an integer of about 1 to about 5;

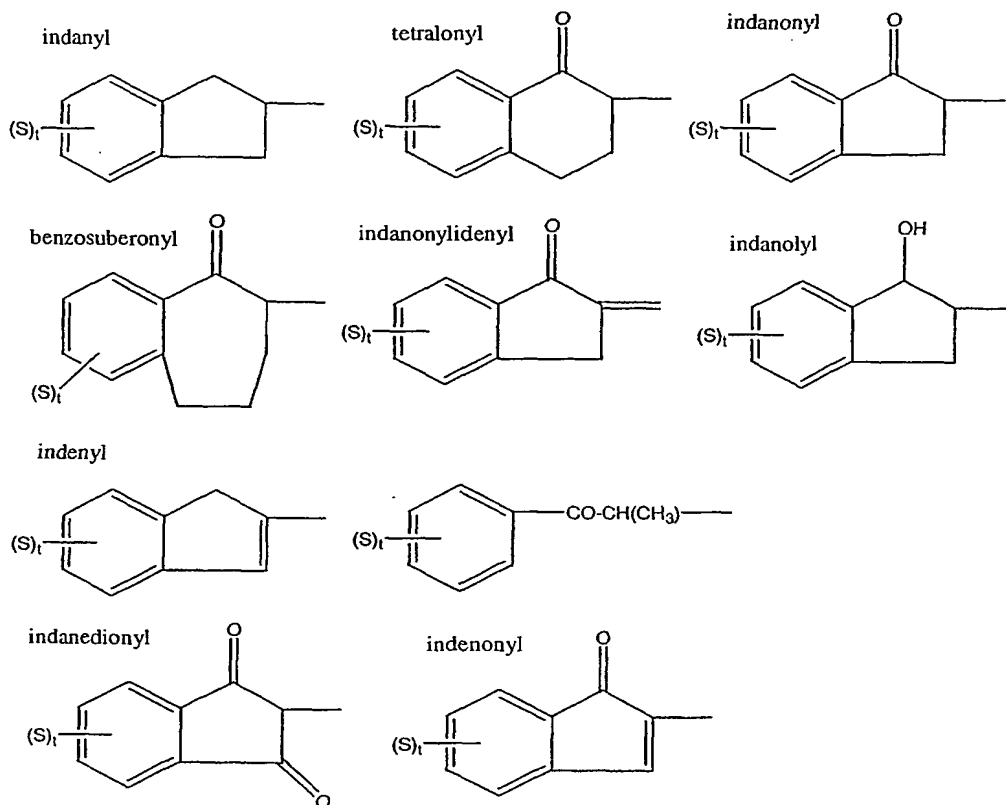
15 T is nitrogen or carbon;



q is an integer of about 1 to about 3;

K is hydrogen, phenyl, substituted phenyl, arylalkyl in which the phenyl can
 have a substituent, cinnamyl, a lower alkyl, pyridylmethyl, cycloalkylalkyl,
 20 adamantanemethyl, furylmenthyl, cycloalkyl, lower alkoxy carbonyl or an acyl; and
 ----- is a single bond or a double bond.

In the compound of formula I, J is preferably (a) or (b), more preferably (b). In
 the definition of (b), a monovalent group (2), (3) and (5) and a divalent group (2) are
 preferred. The group (b) preferably includes, for example, the groups having the
 25 formulae shown below:



wherein t is an integer of about 1 to about 4; and each S is independently hydrogen or a substituent, such as a lower alkyl having 1 to 6 carbon atoms or a lower alkoxy having 1 to 6 carbon atoms. Among the substituents, methoxy is most preferred. The phenyl is most preferred to have 1 to 3 methoxy groups thereon. $(S)_t$ can form methylene dioxy groups or ethylene dioxy groups on two adjacent carbon atoms of the phenyl group. Of the above groups, indanonyl, indanedionyl and indenyl, optionally having substituents on the phenyl, are the most preferred.

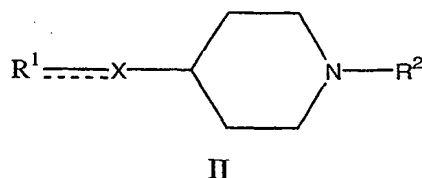
In the definition of B , $-(CHR^{22})_r-$, $-\text{CO}-(CHR^{22})_r-$, $=(\text{CH}-\text{CH}=\text{CH})_b-$, $=\text{CH}-(\text{CH}_2)_c-$ and $=(\text{CH}-\text{CH})_d=$ are preferable. The group of $-(CHR^{22})_r-$ in which R^{22} is hydrogen and r is an integer of 1 to 3, and the group of $=\text{CH}-(\text{CH}_2)_c-$ are most preferable. The preferable groups of B can be connected with (b) of J , in particular (b)(2).

The ring containing T and Q in formula I can be 5-, 6- or 7-membered. It is preferred that Q is nitrogen, T is carbon or nitrogen, and q is 2; or that Q is nitrogen, T is carbon, and q is 1 or 3; or that Q is carbon, T is nitrogen and q is 2.

It is preferable that K is a phenyl, arylalkyl, cinnamyl, phenylalkyl or a

phenylalkyl having a substituent(s) on the phenyl.

In another embodiment, the cyclic amine compounds of formula I are the piperidine compounds of formula II, a stereoisomer thereof, and/or a pharmaceutically acceptable salt thereof:



wherein R¹ is a (1) substituted or unsubstituted phenyl group; (2) a substituted or unsubstituted pyridyl group; (3) a substituted or unsubstituted pyrazyl group; (4) a substituted or unsubstituted quinolyl group; (5) a substituted or unsubstituted indanyl group; (6) a substituted or unsubstituted cyclohexyl group; (7) a substituted or unsubstituted quinoxalyl group; (8) a substituted or unsubstituted furyl group; (9) a monovalent or divalent group derived from an indanone having a substituted or unsubstituted phenyl ring; (10) a monovalent group derived from a cyclic amide compound; (11) a lower alkyl group; or (12) a group of the formula R³-CH=C-, where

15 R³ is a hydrogen atom or a lower alkoxy carbonyl group;

X is -(CH₂)_n-, -C(O)-(CH₂)_n-, -N(R⁴)-(CH₂)_n-, -C(O)-N(R⁵)-(CH₂)_n-, -CH=CH-(CH₂)_n-, -O-C(O)-O-(CH₂)_n-, -O-C(O)-NH-(CH₂)_n-, -CH=CH-CH=CO-, -NH-C(O)-(CH₂)_n-, -CH₂-C(O)-NH-(CH₂)_n-, -(CH₂)₂-C(O)-NH-(CH₂)_n-, -CH(OH)-(CH₂)_n-, -C(O)-CH=CH-CH₂-, -C(O)-CH₂-CH(OH)-CH₂-,

20 -CH(CH₃)-C(O)-NH-CH₂-, -CH=CH-C(O)-NH-(CH₂)₂-, a dialkylaminoalkyl carbonyl group, a lower alkoxy carbonyl group;

where n is an integer of 0 to 6; R⁴ is a hydrogen atom, a lower alkyl group, an acyl group, a lower alkylsulfonyl group, a substituted or unsubstituted phenyl group, or a substituted or unsubstituted benzyl group; and R⁵ is a hydrogen atom a lower alkyl group or a phenyl group;

25

R² is a substituted or unsubstituted phenyl group; a substituted or unsubstituted arylalkyl group; a cinnamyl group; a lower alkyl group; a pyridylmethyl group; a cycloalkylalkyl group; an adamantanemethyl group; or a furoylmethyl group; and

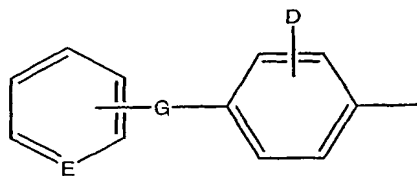
----- is a single bond or a double bond.

30 The term "lower alkyl group" as used herein means a straight or branched alkyl

group having 1 to 6 carbon atoms. Exemplary "lower alkyl groups" include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl (amyl), isopentyl, neopentyl, tert-pentyl, 1-methylbutyl, 2-methylbutyl, 1,2-dimethylpropyl, hexyl, isohexyl, 1-methylpentyl, 2-methyl-pentyl, 3-methylpentyl, 1,1-dimethylbutyl, 1,2-
5 dimethylbutyl, 2,2-dimethylbutyl, 1,3-dimethyl-butyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl, 1-ethyl-2-methylpropyl, and the like. The lower alkyl group is preferably methyl, ethyl, propyl or isopropyl; more preferably methyl.

Specific examples of the substituents for the substituted or unsubstituted phenyl,
10 pyridyl, pyrazyl, quinolyl, indanyl, cyclohexyl, quinoxalyl and furyl groups in the definition of R¹ include lower alkyl groups having 1 to 6 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, and tert-butyl groups; lower alkoxy groups corresponding to the above-described lower alkyl groups, such as methoxy and ethoxy groups; a nitro group; halogen atoms, such as chlorine, fluorine and bromine; a
15 carboxyl group; lower alkoxycarbonyl groups corresponding to the above-described lower alkoxy groups, such as methoxycarbonyl, ethoxycarbonyl, isopropoxycarbonyl, n-propoxycarbonyl, and n-butyloxycarbonyl groups; an amino group; a lower monoalkylamino group; a lower dialkylamino group; a carbamoyl group; acylamino groups derived from aliphatic saturated monocarboxylic acids having 1 to 6 carbon
20 atoms, such as acetylaminio, propionylaminio, butyrylaminio, isobutyrylaminio, valerylaminio, and pivaloylaminio groups; cycloalkyloxycarbonyl groups, such as a cyclohexyloxycarbonyl group; lower alkylaminocarbonyl groups, such as methylaminocarbonyl and ethylaminocarbonyl groups; lower alkylcarbonyloxy groups corresponding to the above-defined lower alkyl groups, such as methylcarbonyloxy,
25 ethylcarbonyloxy, and n-propylcarbonyloxy groups; halogenated lower alkyl groups, such as a trifluoromethyl group; a hydroxyl group; a formyl group; and lower alkoxy lower alkyl groups, such as ethoxymethyl, methoxymethyl and methoxyethyl groups. The "lower alkyl groups" and "lower alkoxy groups" in the above description of the substituent include all the groups derived from the above-mentioned groups. The
30 substituent can be one to three of them, which can be the same or different.

When the substituent is a phenyl group, the following group is within the scope of the substituted phenyl group:



wherein G is -C(O)-, -O-C(O)-, -O-, -CH₂-NH-C(O)-, -CH₂-O-, -CH₂-SO₂-, -CH(OH)-, or -CH₂-S(→O)-; E is a carbon or nitrogen atom; and D is a substituent.

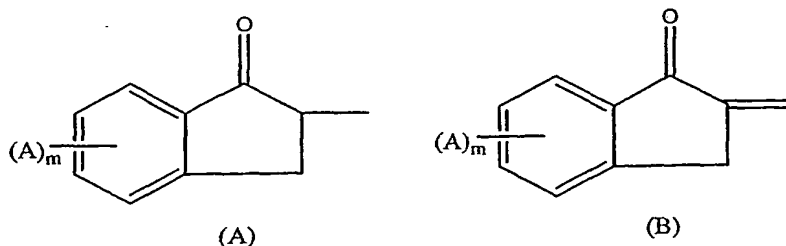
Preferred examples of the substituents (i.e., "D") for the phenyl group include
 5 lower alkyl, lower alkoxy, nitro, halogenated lower alkyl, lower alkoxy carbonyl, formyl, hydroxyl, and lower alkoxy lower alkyl groups, halogen atoms, and benzyl and benzylsulfonyl groups. The substituent can be two or more of them, which can be the same or different.

Preferred examples of the substituent for the pyridyl group include lower alkyl
 10 and amino groups and halogen atoms.

Preferred examples of the substituent for the pyrazyl group include lower alkoxy carbonyl, carboxyl, acylamino, carbamoyl, and cycloalkyloxy carbonyl groups.

With respect to R¹, the pyridyl group is preferably a 2-pyridyl, 3-pyridyl, or 4-pyridyl group; the pyrazyl group is preferably a 2-pyrazinyl group; the quinolyl group
 15 is preferably a 2-quinolyl or 3-quinolyl group; the quinoxaliny group is preferably a 2-quinoxaliny or 3-quinoxaliny group; and the furyl group is preferably a 2-furyl group.

Specific examples of preferred monovalent or divalent groups derived from an indanone having an unsubstituted or substituted phenyl ring include those represented by formulas (A) and (B):

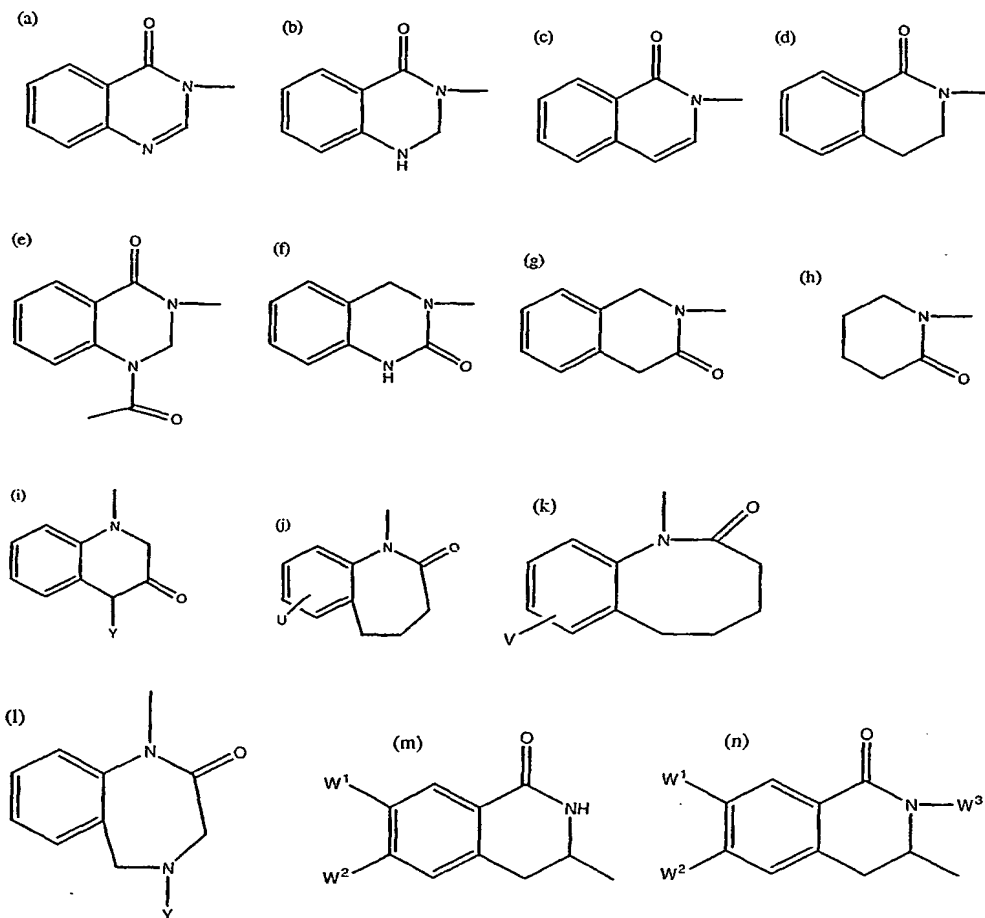


20 where m is an integer of from 1 to 4, and each A is independently a hydrogen atom, a lower alkyl group, a lower alkoxy group, a nitro group, a halogen atom, a carboxyl group, a lower alkoxy carbonyl group, an amino group, a lower monoalkylamino group, a lower dialkylamino group, a carbamoyl group, an acylamino
 25 group derived from aliphatic saturated monocarboxylic acids having 1 to 6 carbon

atoms, a cycloalkyloxycarbonyl group, a lower alkylaminocarbonyl group, a lower alkylcarbonyloxy group, a halogenated lower alkyl group, a hydroxyl group, a formyl group, or a lower alkoxy lower alkyl group; preferably a hydrogen atom, a lower alkyl group or a lower alkoxy group; most preferably the indanone group is unsubstituted or substituted with 1 to 3 methoxy groups.

Examples of the monovalent group derived from a cyclic amide compound include quinazolone, tetrahydroisoquinolinone, tetrahydrobenzodiazepinone, and hexahydrobenzazocinone. However, the monovalent group can be any one having a cyclic amide group in the structural formula thereof, and is not limited to the above-described specific examples. The cyclic amide group can be one derived from a monocyclic or condensed heterocyclic ring. The condensed heterocyclic ring is preferably one formed by condensation with a phenyl ring. In this case, the phenyl ring can be substituted with a lower alkyl group having 1 to 6 carbon atoms, preferably a methyl group, or a lower alkoxy group having 1 to 6 carbon atoms, preferably a methoxy group.

Preferred examples of the monovalent group include the following:



In the above formulae, Y is a hydrogen atom or a lower alkyl group; V and U are each a hydrogen atom or a lower alkoxy group (preferably dimethoxy); W¹ and W² are each a hydrogen atom, a lower alkyl group, or a lower alkoxy group; and W³ is a hydrogen atom or a lower alkyl group. The right hand ring in formulae (j) and (l) is a 7-membered ring, while the right hand ring in formula (k) is an 8-membered ring.

The most preferred examples of the above-defined R¹ include a monovalent group derived from an indanone having an unsubstituted or substituted phenyl group and a monovalent group derived from a cyclic amide compound.

The most preferred examples of the above-defined X include $-(CH_2)_n-$, an amide group, or groups represented by the above formulae where n is 2. Thus, it is most preferred that any portion of a group represented by the formula R¹-----X----- have a carbonyl or amide group.

The substituents involved in the expressions "a substituted or unsubstituted phenyl group" and "a substituted or unsubstituted arylalkyl group" in the above

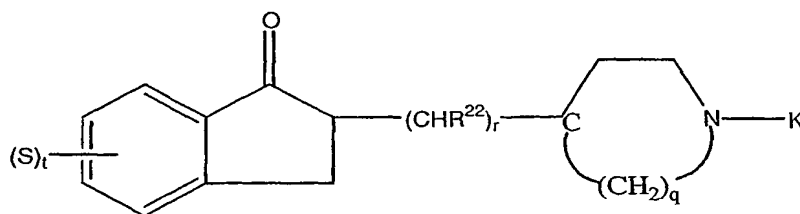
definition of R^2 are the same substituents as those described for the above definitions of a phenyl group, a pyridyl group, a pyrazyl group, a quinolyl group, an indanyl group, a cyclohexyl group, a quinoxalyl group or a furyl group in the definition of R^1 .

The term "arylalkyl group" is intended to mean an unsubstituted benzyl or phenethyl group or the like.

Specific examples of the pyridylmethyl group include 2-pyridylmethyl, 3-pyridylmethyl, and 4-pyridylmethyl groups.

Preferred examples of R^2 include benzyl and phenethyl groups. The symbol $\text{---}\text{---}$ means a double or single bond. The bond is a double bond only when R^1 is the divalent group (B) derived from an indanone having an unsubstituted or substituted phenyl ring, while it is a single bond in other cases.

In another embodiment, the compound of formula II is a compound of formula III, a stereoisomer thereof, and/or a pharmaceutically acceptable salt thereof:



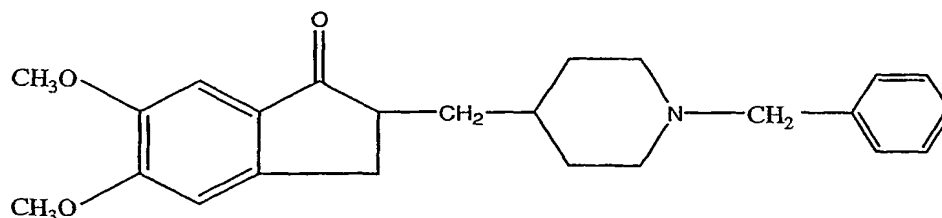
III

wherein r is an integer of about 1 to about 10; each R^{22} is independently hydrogen or methyl; K is a phenalkyl or a phenalkyl having a substituent on the phenyl ring; each S is independently a hydrogen, a lower alkyl group having 1 to 6 carbon atoms or a lower alkoxy group having 1 to 6 carbon atoms; t is an integer of 1 to 4; q is an integer of about 1 to about 3; with the proviso that $(S)_t$ can be a methylenedioxy group or an ethylenedioxy group joined to two adjacent carbon atoms of the phenyl ring.

In other embodiments, the compound of formula III is 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine; 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-ylidenyl)methylpiperidine; 1-benzyl-4-((5-methoxy-1-indanon)-2-yl)methylpiperidine; 1-benzyl-4-((5,6-diethoxy-1-indanon)-2-yl)methylpiperidine; 1-benzyl-4-((5,6-methylenedioxy-1-indanon)-2-yl)methylpiperidine; 1-(*m*-nitrobenzyl)-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine; 1-cyclohexylmethyl-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine; 1-(*m*-fluorobenzyl)-4-((5,6-dimethoxy-1-

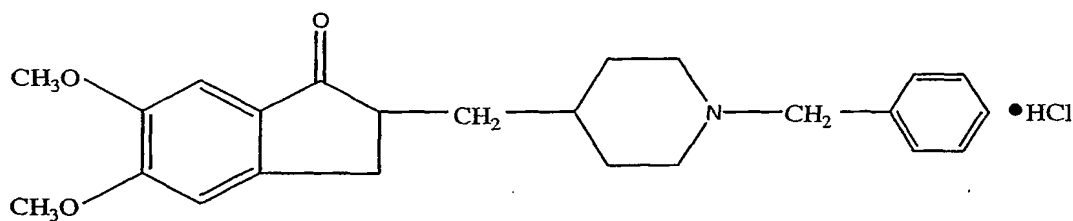
indanon)-2-yl)methylpiperidine; 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-yl)propylpiperidine; 1-benzyl-4-((5-isopropoxy-6-methoxy-1-indanon)-2-yl)methylpiperidine; 1-benzyl-4-((5,6-dimethoxy-1-oxoindanon)-2-yl)propenylpiperidine; or a stereoisomer and/or a pharmaceutically acceptable salt thereof.

In still other embodiments, the compound of formula III is 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, a stereoisomer thereof and/or a pharmaceutically acceptable salt thereof, which is represented by formula IV:



IV.

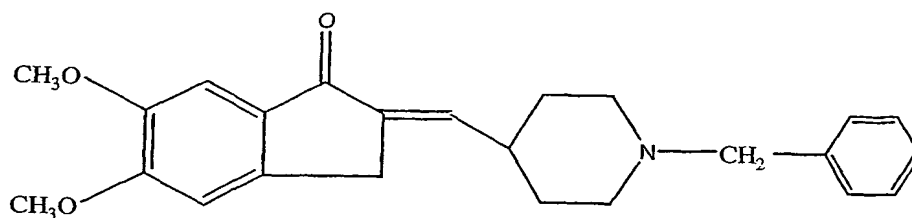
In still other embodiments, the compound of formula III is 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine hydrochloride or a stereoisomer thereof, which is also known as donepezil hydrochloride or ARICEPT® (Eisai Inc., Teaneck, NJ), and which is represented by formula IVa:



IVa.

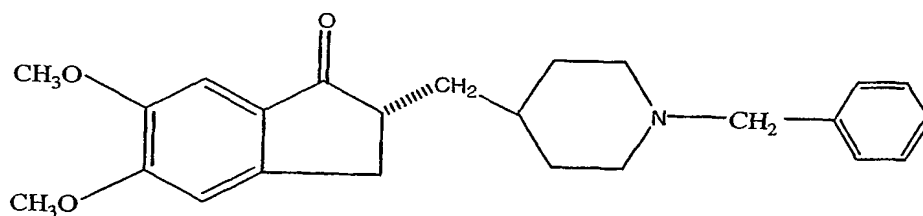
The compounds of the invention can have an asymmetric carbon atom(s), depending upon the substituents, and can have stereoisomers, which are within the scope of the invention. For example, donepezil or pharmaceutically acceptable salts thereof can be in the forms described in Japanese Patent Application Nos. 4-187674 and 4-21670, the disclosures of which are incorporated by reference herein in their entirety.

Japanese Patent Application No. 4-187674 describes a compound of formula V:



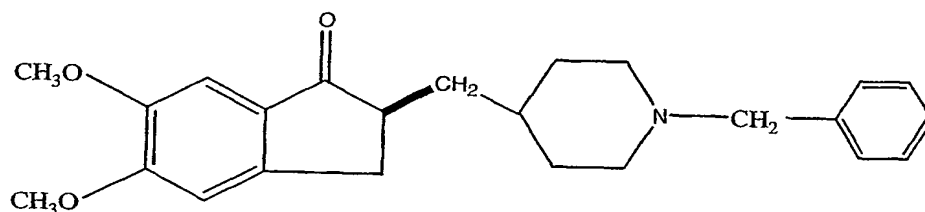
V

which can be in the form of a pharmaceutically acceptable salt, such as a hydrochloride salt. Japanese Patent Application No. 4-21670 describes compounds of formula VI:



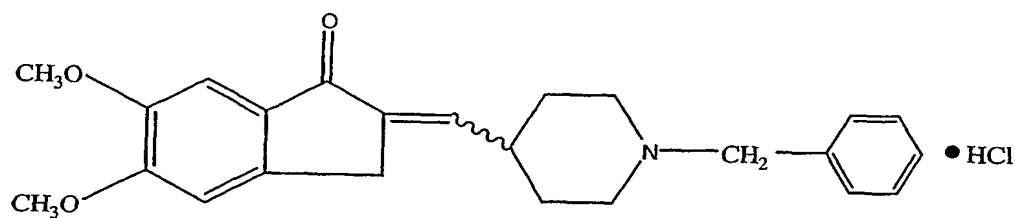
VI

which can be in the form of a pharmaceutically acceptable salt, such as a hydrochloride salt; and compounds of formula VII:



VII

which can be in the form of a pharmaceutically acceptable salt, such as a hydrochloride salt; and compounds of formula VIII:



VIII.

As described above, the cholinesterase inhibitors can be administered in the form of a pharmaceutically acceptable salt. Pharmaceutically acceptable salts are well known in the art and include those of inorganic acids, such as hydrochloride, sulfate,

hydrobromide and phosphate; and those of organic acids, such as formate, acetate, trifluoroacetate, methanesulfonate, benzenesulfonate and toluenesulfonate. When certain substituents are selected, the compounds of the invention can form, for example, alkali metal salts, such as sodium or potassium salts; alkaline earth metal salts, such as calcium or magnesium salts; organic amine salts, such as a salt with trimethylamine, triethylamine, pyridine, picoline, dicyclohexylamine or N,N'-dibenzylethylenediamine. One skilled in the art will recognize that the compounds of the invention can be made in the form of any other pharmaceutically acceptable salt.

The cholinesterase inhibitors can be prepared by processes that are known in the art and described, for example, in U.S. Patent No. 4,895,841, WO 98/39000, and Japanese Patent Application Nos. 4-187674 and 4-21670, the disclosures of each of which are incorporated by reference herein in their entirety. Donepezil hydrochloride, a preferred cholinesterase inhibitor for use in the methods described herein, is commercially available as ARICEPT® from Eisai Inc., Teaneck, NJ.

"Patient" refers to animals, preferably mammals, more preferably humans. The term "patient" includes adults and children, and men and women. In one embodiment, the patient is a human. In another embodiment, the patient is an adult human.

The dosage regimen for treating and preventing the diseases described herein with the cholinesterase inhibitors can be selected in accordance with a variety of factors, including the age, weight, sex, and medical condition of the patient, the route of administration, pharmacological considerations such as the activity, efficacy, pharmacokinetic and toxicology profiles of the cholinesterase inhibitor, whether a drug delivery system is used and whether the cholinesterase inhibitor is administered as part of a drug combination. Dosage regimens for using cholinesterase inhibitors and, optionally, HMG-CoA reductase inhibitors, can also be found by consulting the *Physician's Desk Reference*.

When more than one cholinesterase inhibitor is administered to a patient and/or when the cholinesterase inhibitor(s) is administered in conjunction with another medication (e.g., an HMG-CoA reductase inhibitor, the compounds can be separately administered about the same time as part of an overall treatment regimen, i.e., as a drug cocktail or combination therapy. "About the same time" includes administering the compounds at the same time, at different times on the same day, or on different days, as

long as they are administered as part of an overall treatment regimen.

The cholinesterase inhibitors can be administered to treat or prevent the diseases described herein in doses of about 0.01 milligrams to about 300 milligrams per day; about 1 milligram to about 50 milligrams per day; about 1 milligram to about 25
5 milligrams per day; or about 1 milligram to about 20 milligrams per day. The cholinesterase inhibitor can be administered in an amount of 1 milligram/day; 2 milligrams/day; 3 milligrams/day; 4 milligrams/day; 5 milligrams/day; 6 milligrams/day; 7 milligrams/day; 8 milligrams/day; 9 milligrams/day; 10 milligrams/day; 11 milligrams/day; 12 milligrams/day; 13 milligrams/day; 14
10 milligrams/day; 15 milligrams/day; 16 milligrams/day; 17 milligrams/day; 18 milligrams/day; 19 milligrams/day; 20 milligrams/day; 21 milligrams/day; 22 milligrams/day; 23 milligrams/day; 24 milligrams/day; or 25 milligrams/day. The doses can be administered in one to four portions over the course of a day, preferably once a day.

15 In other embodiments of the methods described herein, donepezil hydrochloride, which is commercially available as ARICEPT® (Eisai Inc., Teaneck, NJ), can be administered as tablets containing either 5 milligrams donepezil hydrochloride or 10 milligrams donepezil hydrochloride. The tablets can be administered one to about four times a day. In one embodiment, one 5 milligram or
20 one 10 milligram ARICEPT® tablet is administered once a day for the methods described herein.

The cholinesterase inhibitors can be administered orally, topically, parenterally, by inhalation (nasal or oral), or rectally in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as
25 desired. Preferably, the cholinesterase inhibitors are orally administered as tablets.

Injectable preparations include subcutaneous, intraarterial, intravenous, intramuscular, intrathecal, intrasternal, infusion techniques, and the like. Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, of the cholinesterase inhibitors can be formulated according to the art using suitable
30 dispersing or wetting agents, suspending agents (e.g., methylcellulose, Polysorbate 80, hydroxyethylcellulose, acacia, powdered tragacanth, sodium carboxymethylcellulose, polyoxyethylene sorbitan monolaurate and the like), pH modifiers, buffers, solubilizing

agents (e.g., polyoxyethylene hydrogenated castor oil, Polysorbate 80, nicotinamide, polyoxyethylene sorbitan monolaurate, Macrogol, an ethyl ester of castor oil fatty acid, and the like) and preservatives. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be used are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally used as a solvent or suspending medium. For this purpose any bland fixed oil can be used including synthetic mono- or diglycerides, in addition, fatty acids, such as oleic acid, can be used in the preparation of injectables. The preparations can be lyophilized by methods known in the art.

In one embodiment, the cholinesterase inhibitors of the invention can be administered to the patient by a spinal pump (e.g., spinal fluid injector pump). In a spinal pump, the medication is administered (e.g., infused, injected) to the patient's spinal cord area. For example, the medication can be administered into the intrathecal space around the spinal cord. Spinal pumps are known in the art and described for example, in U.S. Patent No. 6,682,508, the disclosure of which is incorporated by reference herein in its entirety.

Solid dosage forms for oral administration of the cholinesterase inhibitors can include chewing gum, capsules, tablets, sublingual tablets, powders, granules, and gels; preferably tablets. In such solid dosage forms, the active compound can be admixed with one or more inert diluents such as lactose or starch. As is normal practice, such dosage forms can also comprise other substances including lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms can also comprise buffering agents. The tablets can be prepared with enteric or film coatings, preferably film coatings.

Sublingual administration refers to the administration of the cholinesterase inhibitors in the mouth (e.g., under the tongue, between the cheek and gum, between the tongue and roof of the mouth). The highly vascular mucosal lining in the mouth is a convenient location for the cholinesterase inhibitors to be administered into the body. To make tablets, the cholinesterase inhibitors can be admixed with pharmaceutically acceptable carriers known in the art such as, for example, vehicles (e.g., lactose, white

sugar, mannitol, glucose, starches, calcium carbonate, crystalline cellulose, silicic acid, and the like), binders (e.g., water, ethanol, myranol, glucose solution, starch solution, gelatin solution, polyvinylpyrrolidone, and the like), disintegrators (e.g., dry starch, sodium, alginate, sodium hydrogen carbonate, calcium carbonate, polyoxyethylene sorbitan fatty acid esters, sodium laurylsulfate, stearic monoglyceride, starches, lactose, and the like), absorption promoters (e.g., quaternary ammonium base, sodium laurylsulfate, and the like), wetting agents (e.g. glycerin, starches, and the like), lubricants (e.g., stearates, polyethylene glycol, and the like), and flavoring agents (e.g., sweeteners). The tablets can be in the form of a conventional tablet, a molded tablet, a wafer and the like.

In other embodiments, the solid dosage form can be packaged as granules or a powder in a pharmaceutically acceptable carrier, where the granules or powder are removed from the packaging and sprinkled on food or mixed with a liquid, such as water or juice. In this embodiment, the cholinesterase inhibitors can be mixed with flavoring or sweetening agents. The packaging material can be plastic, coated paper, or any material that prevents water or moisture from reaching the granules and/or powder.

Liquid dosage forms for oral administration of the cholinesterase inhibitors can include pharmaceutically acceptable emulsions, solutions, sublingual solutions, suspensions, and syrups containing inert diluents commonly used in the art, such as water. Such compositions can also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents. To make sublingual solutions, the cholinesterase inhibitors can be admixed with various carriers, excipients, pH adjusters, and the like (e.g., water, sugar, lactic acid, acetic acid, fructose, glucose, saccharin, polyethylene glycol, propylene glycol, alcohol, bentonite, tragacanth, gelatin, alginates, aspartame, sorbitol, methylparaben, propylparaben, sodium benzoate, artificial flavoring and coloring agents).

For administration by inhalation, the cholinesterase inhibitors can be delivered from an insufflator, a nebulizer or a pressured pack or other convenient mode of delivering an aerosol spray. Pressurized packs can include a suitable propellant. Alternatively, for administration by inhalation, the cholinesterase inhibitors can be administered in the form of a dry powder composition or in the form of a liquid spray.

Suppositories for rectal administration of the cholinesterase inhibitors can be

prepared by mixing the active compounds with suitable nonirritating excipients such as cocoa butter and polyethylene glycols that are solid at room temperature and liquid at body temperature. Alternatively, an enema can be prepared by for rectal administration of the cholinesterase inhibitors.

5 For topical administration to the epidermis, the cholinesterase inhibitors can be formulated as ointments, creams or lotions, or as the active ingredient of a transdermal patch. The cholinesterase inhibitors can also be administered via iontophoresis or osmotic pump. Ointments, creams and lotions can be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Alternatively,
10 ointments, creams and lotions can be formulated with an aqueous or oily base and can also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, and/or coloring agents. As creams or lotions, the cholinesterase inhibitors can be mixed to form a smooth, homogeneous cream or lotion with, for example, one or more of a preservative (e.g., benzyl alcohol 1% or 2%
15 (wt/wt)), emulsifying wax, glycerin, isopropyl palmitate, lactic acid, purified water, sorbitol solution. Such topically administrable compositions can contain polyethylene glycol 400. To form ointments, the cholinesterase inhibitors can be mixed with one or more of a preservative (e.g., benzyl alcohol 2% (wt/wt)), petrolatum, emulsifying wax, and Tenox (II) (e.g., butylated hydroxyanisole, propyl gallate, citric acid, propylene
20 glycol). Woven pads or rolls of bandaging material, e.g., gauze, can be impregnated with the transdermally administrable compositions for topical application.

The cholinesterase inhibitors can also be topically applied using a transdermal system, such as one of an acrylic-based polymer adhesive with a resinous crosslinking agent impregnated with the cholinesterase inhibitors and laminated to an impermeable
25 backing. For example, the cholinesterase inhibitors can be administered in the form of a transdermal patch, such as a sustained-release transdermal patch. Transdermal patches can include any conventional form such as, for example, an adhesive matrix, a polymeric matrix, a reservoir patch, a matrix- or monolithic-type laminated structure, and are generally comprised of one or more backing layers, adhesives, penetration
30 enhancers, and/or rate-controlling membranes. Transdermal patches generally have a release liner which is removed to expose the adhesive/active ingredient(s) prior to application. Transdermal patches are described in, for example, U.S. Patent Nos.

5,262,165, 5,948,433, 6,010,715 and 6,071,531, the disclosures of which are incorporated by reference herein in their entirety.

5 The invention provides for the cholinesterase inhibitors to be administered nasally to a patient to treat the diseases and disorders described herein and those described, for example, in PCT/US02/29734, WO 01/66114, and U.S. Patent Nos. 6,482,838, 6,458,807 and 6,455,544, the disclosures of which are incorporated by reference herein in their entirety. "Administered nasally" or "nasal administration" is intended to mean that at least one cholinesterase inhibitor is combined with a suitable delivery system for absorption across the nasal mucosa of a patient, preferably a 10 human. Generally, lower doses of the cholinesterase inhibitor can be used for nasal administration when compared, for example, to the dose required for the oral administration of the cholinesterase inhibitor.

The cholinesterase inhibitors of the invention can be administered, for example, as nasal sprays, nasal drops, nasal suspensions, nasal gels, nasal ointments, nasal 15 creams or nasal powders. The cholinesterase inhibitors can also be administered using nasal tampons or nasal sponges. The cholinesterase inhibitors of the invention can be brought into a viscous basis via systems conventionally used, for example, natural gums, methylcellulose and derivatives, acrylic polymers (carbopol) and vinyl polymers (polyvinylpyrrolidone). In the compositions, many other excipients known in the art 20 can be added such as water, preservatives, surfactants, solvents, adhesives, antioxidants, buffers, bio-adhesives, viscosity enhancing agents and agents to adjust the pH and the osmolarity.

The nasal delivery systems can take various forms including aqueous solutions, non-aqueous solutions and combinations thereof. Aqueous solutions include, for 25 example, aqueous gels, aqueous suspensions, aqueous liposomal dispersions, aqueous emulsions, aqueous microemulsions and combinations thereof. Non-aqueous solutions include, for example, non-aqueous gels, non-aqueous suspensions, non-aqueous liposomal dispersions, non-aqueous emulsions, non-aqueous microemulsions and combinations thereof.

30 In other embodiments, the nasal delivery system can be a powder formulation. Powder formulations include, for example, powder mixtures, powder microspheres, coated powder microspheres, liposomal dispersions and combinations thereof.

Preferably, the powder formulation is powder microspheres. The powder microspheres are preferably formed from various polysaccharides and celluloses selected from starch, methylcellulose, xanthan gum, carboxymethylcellulose, hydroxypropyl cellulose, carbomer, alginate polyvinyl alcohol, acacia, chitosans, and mixtures of two or more thereof.

In certain embodiments, the particle size of the droplets of the aqueous and/or non-aqueous solution or of the powders delivered to the nasal mucosa can be, for example, about 0.1 micron to about 100 microns; from about 1 micron to about 70 microns; from about 5 microns to about 50 microns; or from about 10 microns to about 20 microns. The particle sizes can be obtained using suitable containers or metering devices known in the art. Exemplary devices include mechanical pumps in which delivery is made by movement of a piston; compressed air mechanisms in which delivery is made by hand pumping air into the container; compressed gas (e.g., nitrogen) techniques in which delivery is made by the controlled release of a compressed gas in the sealed container; liquefied propellant techniques in which a low boiling liquid hydrocarbon (e.g., butane) is vaporized to exert a pressure and force the composition through the metered valve; and the like. Powders may be administered, for example, in such a manner that they are placed in a capsule that is then set in an inhalation or insufflation device. A needle is penetrated through the capsule to make pores at the top and the bottom of the capsule and air is sent to blow out the powder particles. Powder formulation can also be administered in a jet-spray of an inert gas or suspended in liquid organic fluids.

In one embodiment, the invention provides a nasally administrable pharmaceutical composition comprising at least one cholinesterase inhibitor dispersed in a nasal delivery system that improves the solubility of the cholinesterase inhibitor. The nasal delivery system that improves solubility can include one of the following or combinations thereof: (i) a glycol derivative (e.g., propylene glycol, polyethylene glycol, mixtures thereof); (ii) a sugar alcohol (e.g., mannitol, xylitol, mixtures thereof); (iii) glycerin; (iv) a glycol derivative (e.g., propylene glycol, polyethylene glycol or mixtures thereof) and glycerin; (v) ascorbic acid and water; (vi) sodium ascorbate and water; or (vii) sodium metabisulfite and water.

In another embodiment, the invention provides a nasally administrable

pharmaceutical composition comprising at least one cholinesterase inhibitor and a nasal delivery system, where the nasal delivery system comprises at least one buffer to maintain the pH of the cholinesterase inhibitor, at least one pharmaceutically acceptable thickening agent and at least one humectant. The nasal delivery system can optionally further comprise surfactants, preservatives, antioxidants, bio-adhesives, pH adjusting agents, isotonicity agents, solubilizing agents, and/or other pharmaceutically acceptable excipients. The cholinesterase inhibitor can optionally be dispersed in a nasal delivery system that improves its solubility.

In another embodiment, the invention provides a nasally administrable pharmaceutical composition comprising at least one cholinesterase inhibitor and a nasal delivery system, where the nasal delivery system comprises at least one solubilizing agent, at least one pharmaceutically acceptable thickening agent and at least one humectant. The nasal delivery system can optionally further comprise buffers, pH adjusting agents, isotonicity agents, surfactants, preservatives, antioxidants, bio-adhesives, and/or other pharmaceutically acceptable excipients. The cholinesterase inhibitor can optionally be dispersed in a nasal delivery system that improves its solubility.

In another embodiment, the invention provides a nasally administrable pharmaceutical composition comprising at least one cholinesterase inhibitor and a nasal delivery system, where the nasal delivery system comprises at least one buffer to maintain the pH of the cholinesterase inhibitor, at least one pharmaceutically acceptable thickening agent, at least one humectant, and at least one surfactant. The nasal delivery system can optionally further comprise pH adjusting agents, isotonicity agents, solubilizing agents, preservatives, antioxidants, bio-adhesives, and/or other pharmaceutically acceptable excipients. The cholinesterase inhibitor can optionally be dispersed in a nasal delivery system that improves its solubility.

In yet another embodiment, the invention provides a nasally administrable pharmaceutical composition comprising at least one cholinesterase inhibitor and a nasal delivery system, where the nasal delivery system comprises at least one pharmaceutically acceptable thickening agent, at least one humectant, at least one surfactant, and at least one solubilizing agent. The nasal delivery system can optionally further comprise buffers, pH adjusting agents, isotonicity agents, preservatives,

antioxidants, bio-adhesives, and/or other pharmaceutically acceptable excipients. The cholinesterase inhibitor can optionally be dispersed in a nasal delivery system that improves its solubility.

In yet another embodiment, the invention provides a nasally administrable
5 pharmaceutical composition comprising at least one cholinesterase inhibitor and a nasal delivery system, where the nasal delivery system comprises at least one buffer to maintain the pH of the cholinesterase inhibitor, at least one pharmaceutically acceptable thickening agent, at least one humectant, at least one surfactant, and at least one
10 solubilizing agent. The nasal delivery system can optionally further comprise buffers, pH adjusting agents, isotonicity agents, preservatives, antioxidants, bio-adhesives, and/or other pharmaceutically acceptable excipients. The cholinesterase inhibitor can optionally be dispersed in a nasal delivery system that improves its solubility.

The nasally administrable pharmaceutical compositions of the invention preferably provide a peak plasma concentration of the cholinesterase inhibitor in less
15 than one hour, preferably within about 5 minutes to about 30 minutes, more preferably within about 5 minutes to about 20 minutes, after administration to the patient.

The buffer has a pH that is selected to optimize the absorption of the cholinesterase inhibitor across the nasal mucosa. The particular pH of the buffer can vary depending upon the particular nasal delivery formulation as well as the specific
20 cholinesterase inhibitor selected. Buffers that are suitable for use in the invention include acetate (e.g., sodium acetate), citrate (e.g., sodium citrate dihydrate), phthalate, borate, prolamine, trolamine, carbonate, phosphate (e.g., monopotassium phosphate, disodium phosphate), and mixtures of two or more thereof.

The pH of the compositions should be maintained from about 3.0 to about 10.0.
25 Compositions having a pH of less than about 3.0 or greater than about 10.0 can increase the risk of irritating the nasal mucosa of the patient. Further, it is preferable that the pH of the compositions be maintained from about 3.0 to about 9.0. With respect to the non-aqueous nasal formulations, suitable forms of buffering agents can be selected such that when the formulation is delivered into the nasal cavity of a mammal, selected pH
30 ranges are achieved therein upon contact with, e.g., a nasal mucosa.

The solubilizing agent for use in the compositions of the invention can be any known in the art, such as carboxylic acids and salts thereof. Exemplary carboxylic acid

salts include acetate, gluconate, ascorbate, citrate, fumarate, lactate, tartrate, maleate, maleate, succinate, or mixtures of two or more thereof.

The viscosity of the compositions of the present invention can be maintained at a desired level using a pharmaceutically acceptable thickening agent. For example, the
5 viscosity may be at least 1000 cps; from about 1000 to about 10,000 cps; from about 2000 cps to about 6500 cps; or from about 2500 cps to about 5000 cps. Thickening agents that can be used in accordance with the present invention include, for example, methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, polyvinyl alcohol, alginates, acacia, chitosans, and mixtures of two or more
10 thereof. The concentration of the thickening agent will depend upon the agent selected and the viscosity desired. Such agents can also be used in a powder formulation.

The nasally administrable compositions can also include a humectant to reduce or prevent drying of the mucus membrane and to prevent irritation thereof. Suitable humectants that can be used include, for example, sorbitol, mineral oil, vegetable oil
15 and glycerol; soothing agents; membrane conditioners; sweeteners; and mixtures of two or more thereof. The concentration of the humectant will vary depending upon the agent selected. In one embodiment, the humectant can be present in the nasal delivery system in a concentration ranging from about 0.01% to about 20% by weight of the composition.

20 In other embodiments, the nasal delivery system can further comprise surfactants which enhance the absorption of the cholinesterase inhibitor. Suitable surfactants include non-ionic, anionic and cationic surfactants. Exemplary surfactants include oleic acid, polyoxyethylene derivatives of fatty acids, partial esters of sorbitol anhydride, such as for example, Tweens (e.g., Tween 80, Tween 40, Tween 20), Spans
25 (e.g., Span 40, Span 80, Span 20), polyoxyl 40 stearate, polyoxy ethylene 50 stearate, fusicates, bile salts, octoxynol, and mixtures of two or more thereof. Exemplary anionic surfactants include salts of long chain hydrocarbons (e.g., C₆₋₃₀ or C₁₀₋₂₀) having one or more of the following functional groups: carboxylates; sulfonates; and sulfates. Salts of long chain hydrocarbons having sulfate functional groups are
30 preferred, such as sodium cetostearyl sulfate, sodium dodecyl sulfate and sodium tetradecyl sulfate. One particularly preferred anionic surfactant is sodium lauryl sulfate (i.e., sodium dodecyl sulfate). The surfactants can be present in an amount from about

0.001% to about 50% by weight, or from about 0.001% to about 20% by weight.

The pharmaceutical compositions of the invention may further comprise an isotonicity agent, such as sodium chloride, dextrose, boric acid, sodium tartrate or other inorganic or organic solutes.

5 The nasal pharmaceutical compositions of the invention can optionally be used in combination with a pH adjusting agent. Exemplary pH adjusting agents include sulfuric acid, sodium hydroxide, hydrochloric acid, and the like.

10 To extend shelf life, preservatives can be added to the nasally administrable compositions. Suitable preservatives that can be used include benzyl alcohol, parabens, thimerosal, chlorobutanol, benzalkonium chloride, or mixtures of two or more thereof. Preferably benzalkonium chloride is used. Typically, the preservative will be present in a concentration of up to about 2% by weight. The exact concentration of the preservative, however, will vary depending upon the intended use and can be easily ascertained by one skilled in the art.

15 Other ingredients which extend shelf life can be added such as for example, antioxidants. Some examples of antioxidants include sodium metabisulfite, potassium metabisulfite, ascorbyl palmitate and the like. Typically, the antioxidant will be present in the compositions in a concentration of from about 0.001% up to about 5% by weight of the total composition.

20 Other optional ingredients can also be incorporated into the nasal delivery system provided that they do not interfere with the action of the cholinesterase inhibitor or significantly decrease the absorption of the cholinesterase inhibitor across the nasal mucosa.

25 The nasal delivery systems can be made following the processes described in, for example, U.S. Patent Nos. 6,451,848, 6,436,950, and 5,874,450, and WO 00/00199, the disclosures of which are incorporated by reference herein in their entirety.

Each of the patents, patent applications, and publications cited herein are incorporated by reference herein in their entirety.

30 It will be apparent to one skilled in the art that various modifications can be made to the invention without departing from the spirit or scope of the appended claims.

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